Chemistry & Biology
Previews

# Ready, Set, Go! How Protein Kinase C Manages Dynamic Signaling

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http://dx.doi.org/10.1016/j.chembiol.2014.04.003

**In this issue of *Chemistry & Biology*, Antal and colleagues describe how phosphorylation optimizes the signaling range of protein kinase C (PKC) isoforms. Priming of these enzymes regulates intramolecular conformational changes, which reduces access to their diacylglycerol (DAG) binding C1 domains.**

Signaling networks make use of diverse protein modules to communicate signals throughout the cell (Pawson, 2007). A classic example of a multiple module enzyme that mediates various signaling events is the protein kinase C (PKC) family of serine/threonine kinases (Newton, 2010). PKCs regulate diverse cellular processes such as cell growth and differentiation, apoptosis, and learning and memory. There is still a dearth of knowledge on the molecular forces that regulate PKC conformational dynamics and how optimal signaling is tuned. These are important questions to answer, because dysregulation of PKCs can lead to diseases including cancer, diabetes, and neurodegeneration.

The PKC isozymes are separated into three groups based upon the architecture of their membrane-binding domains (Newton, 2010) (Figure 1A). All isozymes have a C-terminal kinase domain, a conserved C-terminal tail sequence that is primed by phosphorylation, and an autoinhibitory pseudosubstrate in the N-terminal region. The pseudosubstrate engages the substrate-binding cleft keeping PKC in an inactive state until DAG binding displaces the inhibition. cPKCs include the $\alpha$, $\beta$, and $\gamma$ isozymes, which harbor N-terminal tandem C1 domains and a C2 domain that binds $Ca^{2+}$, phosphatidylserine, and phosphoinositides. nPKCs comprise $\delta$, $\varepsilon$, $\eta$, and $\theta$ isoforms, which have tandem C1 domains and C2 domains that lack high membrane affinity (Cho and Stahelin, 2006) but can engage phosphotyrosine on target proteins (Benes et al., 2005). Atypical PKCs $\zeta$ and $\iota/\lambda$ do not respond to $Ca^{2+}$ or DAG and undergo protein-protein interactions that regulate cellular function and localization (Kazanietz et al., 1994).

PKCs mature into an active form by a series of phosphorylation events (Newton 2010). Prior to posttranslational modification, newly synthesized PKC is membrane associated (Sonnenburg et al., 2001) but harbors a substrate-binding cleft void of pseudosubstrate (Dutil and Newton, 2000). In absence of phosphorylation, PKC is inactive even in the DAG membrane-bound form (Sonnenburg et al., 2001). Three tightly regulated phosphorylation events of the activation loop, turn motif, and hydrophobic motif are required for optimal PKC catalytic activity (Newton, 2010). Strikingly, the mature PKC is autoinhibited and cytosolic, whereas intramolecular conformational dynamics have changed to restrict PKC DAG and membrane affinity.

A large focus of PKC research over the last two decades has investigated how C1 and C2 domains mediate membrane recruitment and subsequent activation of these enzymes. Some of the key questions in the field have been the mechanism behind formation of intramolecular interactions and how restriction of C1 domain DAG binding occurs. PKC C1 domains (Figure 1B) harbor a DAG-binding pocket that is surrounded by several hydrophobic residues. Isolated C1 domains bind membranes with and without DAG while full-length PKCs that are mature have a lower affinity for DAG containing membranes. Because cPKCs and nPKCs contain two C1 domains (C1A and C1B), studies have investigated which domain is the predominant membrane-binding unit or which domain becomes more restricted in the mature form. Some answers to these questions were recently provided in the X-ray structure of PKCβII, which revealed that the C1B domain acts as a clamp to restrict PKC activity prior to membrane binding (Leonard et al., 2011). However, crystallography of full-length or multimodular PKC structures has been difficult, especially when considering the multiple conformations that would need to be elucidated at different stages of the maturation cycle. Thus, how PKC isozymes are optimally driven toward different levels of DAG has not been well established.

In this issue of *Chemistry & Biology*, several significant questions on PKC regulation and the fine-tuning of C1 domain membrane binding have been answered. Antal et al. (2014) first observed that fluorescently tagged kinase-dead mutations of PKCβII more rapidly translocated to the plasma membrane in response to phorbol dibutyrate, a PKC agonist. To this end, they employed a number of mutations that alter PKC maturation and prevent catalytic competence. In addition to responding more quickly to agonist, these mutations were also moderately localized to plasma membrane prior to agonist addition, strongly suggesting higher membrane affinity than mature wild-type (WT) PKC. The phosphorylation state also wasn't directly correlated with the response rate to agonist addition, because WT and one kinase dead mutation (D466N, which is the catalytic base in the phosphorylation reaction) had comparable levels of phosphorylation. Instead, the phosphorylation order and conformational transitions are important and cannot be mimicked in the catalytically inactive form.

To dig deeper into the mechanism of PKC conformational dynamics, Newton

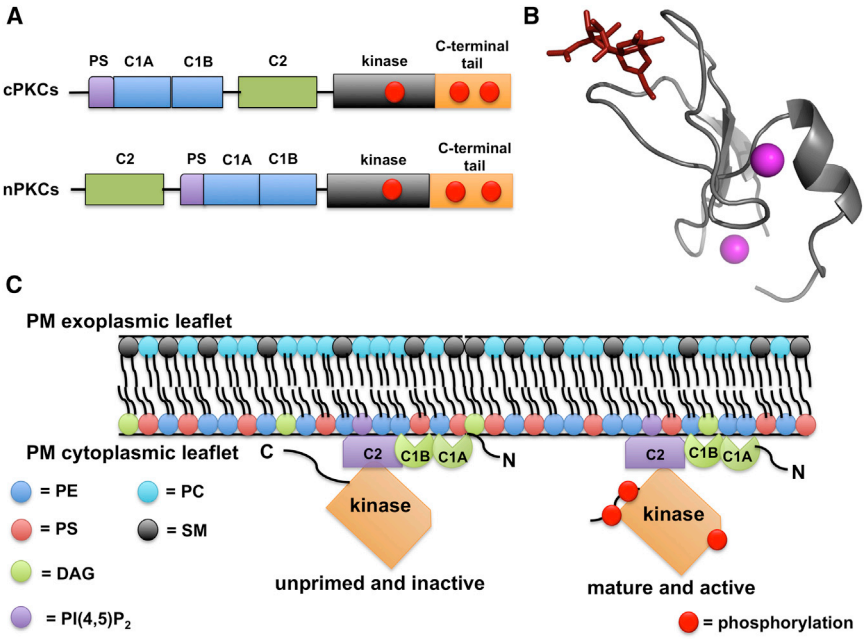


**Figure 1. PKC Architecture and Conformational Dynamics**

(A) The architecture of cPKCs and nPKCs is shown. cPKCs have an N-terminal autoinhibitory pseudosubstrate (PS) followed by the C1A and C1B domains. Between the tandem C1 domains and catalytic domain lies the $Ca^{2+}$- and membrane-binding C2 domain. The kinase and C-terminal tail region harbor phosphorylation sites that include (from N to C terminus) the activation loop, turn motif, and hydrophobic motif (Newton, 2010). Phosphorylation of these sites primes PKC for catalytic activity.

(B) The C1B domain of PKCδ is shown in complex with phorbol-13-acetate (red) (Protein Data Bank ID: 1PTR). Two zinc ions required for structural stability are shown in magenta. For PKCβII, Newton and colleagues demonstrate that the C1B domain is dominant in the mature form.

(C) PKC maturation induces conformational changes that restrict C1 domain membrane and DAG binding. In newly synthesized PKC, the C1A and C1B domains are exposed and promote high membrane affinity. However, in the absence of phosphorylation, the enzyme is not active. Maturation via phosphorylation restricts the C1 domain accessibility in full-length PKC, leading to optimal sensing of DAG levels. In mature and membrane bound cPKC, the C2 domain is engaged in $Ca^{2+}$-dependent interactions with phosphatidylserine and $PI(4,5)P_2$ while the C1 domains bind DAG. DAG binding induces release of the autoinhibitory pseudosubstrate promoting PKC activation.

(A) and (C) were adapted from Antal et al.( 2014).

and colleagues generated several new PKC constructs for live cell imaging (Antal et al., 2014). A FRET-based reporter was engineered to monitor PKC conformational transitions under various cellular conditions. A CFP was encoded at the N terminus of PKC while YFP was introduced at the PKC C terminus. This construct was called Kinameleon and undergoes FRET when CFP and YFP are in close proximity. Essentially, in the unprimed PKC, the CFP and YFP are farther apart as PKCs modular domains are liberated, yielding a low level of FRET. As PKC maturation ensues, FRET increases as phosphorylation induces conformational rearrangement of the N- and C-terminal regions of PKC, bringing the CFP and YFP in close proximity. Although a large portion of the work was done with PKCβII constructs, the authors also generated several constructs in the α and δ isozymes, revealing that PKC maturation is likely a global mechanism regulating PKC conformational changes and sensitivity to DAG.

To investigate if C1A and C1B domains are exposed or masked in primed and unprimed PKCs, the authors generated several constructs to study full-length PKC and the isolated tandem C1 domains. They also asked the question, which C1 domain predominates in membrane binding? Newly synthesized PKC has exposed C1A and C1B domains prior to phosphorylation, which explains the higher membrane affinity, prelocalization at membranes, and rapid response upon agonist addition. Priming phosphorylation then acts to mask both C1A and C1B domains through intramolecular interactions. Although both of these C1 domains contribute to membrane binding of PKCβII, the C1B plays a dominant role. The restriction of C1 domains in mature PKC allows for high sensitivity to small changes in DAG levels, providing different PKC isoforms a means to respond to DAG signals based upon their C1 and C2 domain architecture.

Interestingly, Newton and colleagues (Antal et al., 2014) demonstrate that PKC undergoes conformational changes during the maturation process in live cells. They have also answered several burning questions regarding C1 domain accessibility and PKC membrane interactions. This innovative and high impact work should redefine the way we view PKC regulation and response to different agonists. These studies are also critical to our understanding of how to pharmacologically target this important class of enzymes (Blumberg et al., 2008). What the precise intramolecular interactions that occur in different PKC isoforms are still unknown and may include C2-C1, C1-C1, and C1-C terminus interactions. Nonetheless, these details can now be investigated using the tools Newton and colleagues have generated (Antal et al., 2014). This study should serve as a model for how other multimodular enzymes or complexes may function.

## REFERENCES

Antal, C.E., Violin, J.D., Kunkel, M.T., Skovsø, S., and Newton, A.C. (2014). Chem. Biol. *21*, this issue, 459–469.

Benes, C.H., Wu, N., Elia, A.E., Dharia, T., Cantley, L.C., and Soltoff, S.P. (2005). Cell *121*, 271–280.

Blumberg, P.M., Kedei, N., Lewin, N.E., Yang, D., Czifra, G., Pu, Y., Peach, M.L., and Marquez, V.E. (2008). Curr. Drug Targets *9*, 641–652.

Cho, W., and Stahelin, R.V. (2006). Biochim. Biophys. Acta *1761*, 838–849.

Dutil, E.M., and Newton, A.C. (2000). J. Biol. Chem. *275*, 10697–10701.

Kazanietz, M.G., Bustelo, X.R., Barbacid, M., Kolch, W., Mischak, H., Wong, G., Pettit, G.R., Bruns, J.D., and Blumberg, P.M. (1994). J. Biol. Chem. *269*, 11590–11594.

Leonard, T.A., Różycki, B., Saidi, L.F., Hummer, G., and Hurley, J.H. (2011). Cell *144*, 55–66.

Newton, A.C. (2010). Am. J. Physiol. Endocrinol. Metab. *298*, E395–E402.

Pawson, T. (2007). Curr. Opin. Cell Biol. *19*, 112–116.

Sonnenburg, E.D., Gao, T., and Newton, A.C. (2001). J. Biol. Chem. *276*, 45289–45297.

 